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The Missing Link: Mul1 Signals Mitophagy and Muscle Wasting

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FoxO3 regulates the transcription of autophagy-related genes that induce mitophagy in muscle wasting. In this issue of *Cell Metabolism*, Lokireddy et al. (2012) now show that the mitochondrial ubiquitin protein ligase 1 (Mul1) polyubiquitinates the mitochondrial fusion protein Mfn2 and is necessary for FoxO3- and lysosomal-dependent mitophagy in muscle atrophy.

Free amino acids are not stored in the body. Instead, tissue proteins can be mobilized by enhanced proteolysis to generate high amounts of these precursors of protein synthesis and other molecules like purines and pyrimidines. Skeletal muscle comprises about 40% of whole-body protein, and this reservoir is mobilized into free amino acids under a number of wasting conditions that include deficient protein or energy intake (starvation), reduced physical activity or bedrest, and numerous pathological states. More importantly, muscle wasting is observed in diseases that are highly prevalent both in Western and developing countries and are responsible for high rates of mortality. These include—but are not limited to—the majority of advanced cancers (Fearon et al., 2012), cardiovascular diseases, chronic obstructive pulmonary disease, diabetes, and renal failure. Short-term wasting is highly beneficial: free amino acids arising from the enhanced breakdown of muscle proteins are mostly used to provide energy by direct oxidation and neoglucogenesis. Amino acids are also used for the immune response and for preserving protein synthesis in obligatory working organs (heart, brain, diaphragm, etc.). By contrast, prolonged wasting is a vicious cycle resulting in a debilitating syndrome that comprises loss of strength and weakness, decreased ability to recover, impaired efficiency of treatments, and ultimately death. Therefore, understanding the mechanisms responsible for muscle wasting is critical for both reducing health care costs and improving survival. In the present issue of *Cell Metabolism*, Lokireddy et al. (2012) provide insights into Mul1, a key protein that promotes mitophagy and skeletal muscle loss.

In previous work, Romanello et al. (2010) demonstrated that mitochondria, which were suspected to trigger catabolic signals, contribute to muscle loss and weakness. The authors showed that changes in mitochondrial network prevail in atrophying muscles from fasted and denervated mice. Expression of the mitochondrial fission machinery per se induces muscle wasting in such conditions by triggering organelle dysfunction and AMPK activation (Figure 1). Conversely, inhibition of mitochondrial fission inhibits muscle loss during fasting. The authors also demonstrated that FoxO3 overexpression induces mitochondrial disruption through activation of autophagy. These data were in perfect agreement with another paper (Mammucari et al., 2007), which demonstrated that FoxO3 induces autophagy and stimulates expression of many autophagy (Atg) genes in myotubes, in isolated muscle fibers and in muscle in vivo. Furthermore, Akt/PKB activation blocks FoxO3 activation and autophagy (Figure 1).

In this issue of *Cell Metabolism*, Lokireddy et al. (2012) provide elegant and strong evidence that the mitochondrial E3 ubiquitin protein ligase 1 (Mul1, also called MAPL, C1orf166, GIDE, MULAN, or RNF218) is sufficient for inducing mitophagy and muscle wasting. First the authors examined the expression of known regulators of mitochondrial dynamics in catabolic myotubes. These C2C12 cells have been either starved or exposed to inducers of muscle wasting such as dexamethasone (Dex, a synthetic glucocorticoid) or recombinant human myostatin (hMstn). They identify various genes involved in mitochondrial metabolism or autophagy that are upregulated in such conditions, including Mul1. Since Mul1 was already reported to play a role in mitochondrial fission (Braschi et al., 2009), the authors next verified that starvation- and denervation-induced muscle atrophy also results in increased levels of Mul1 in mouse muscle in vivo. They then identified six putative FoxO transcription factor binding sites in the human Mul1 promoter and showed that FoxO3 interacts physically with the Mul1 promoter. Accordingly, Mul1 promoter activity increased in the presence of constitutively active FoxO3 (ca-FoxO3) in Dex, hMstn, and starved myotubes, and Mul1 protein levels increased in C2C12 myotubes and in ca-FoxO3 starved muscles.

Importantly, Mul1 siRNA in catabolic myoblasts and shRNA in the starved tibialis anterior muscle blocked mitophagy. The authors then identified the mitochondrial fusion protein Mitofusin-2 (Mfn2) as a target of Mul1 and showed that polyubiquitinated Mfn2, or a high ratio of polyubiquitinated Mfn2/total Mfn2 (Figure 1), correlates with mitophagy. Indeed, Mul1 siRNA increased functional mitochondria in Dex- and hMstn-treated and starved C2C12 myoblasts. Moreover, the proportion of mitochondria internalized into phagosomes increased in Dex- and hMstn-treated or starved C2C12 cells, but this adaptation was reversed by Mul1 siRNA in the three catabolic states. Finally, GFP-Mul1 colocalizes with RFP-LC3, a marker of authophagy. LC3II (the lipidated and active form of LC3) increases in all catabolic conditions studied in vitro, and that adaptation is blocked by Mul1 shRNA in atrophying muscles.

Altogether these data nicely demonstrate that Mul1 is critical for the inhibition of mitochondrial fusion through the autophagic pathway and for muscle wasting. Thus, Mul1-dependent polyubiquitination of Mfn2 does not affect the breakdown of muscle proteins per se. This situation is analogous to findings regarding enhanced expression of ubiquitination and deubiquitination enzymes, which also prevails in muscle wasting. The overexpressed muscle-specific E3 MAFbx/atrogin-1 and deubiquitinating enzyme USP19 were believed to be markers of enhanced proteolysis but are more related to depressed protein synthesis (reviewed in Attaix et al. [2012]). Signaling pathways of protein synthesis and breakdown are thus intricate and interdependent, acting through key proteins at their crossroads (i.e., Akt that is activated by anabolic stimuli, see Figure 1, or the glucocorticoid receptor, see Shimizu et al. [2011]). So far, only MuRF1, another muscle-specific E3, has been shown to directly target major contractile muscle proteins for breakdown by the ubiquitin-proteasome system (UPS) (Clarke et al., 2007; Polge et al., 2011).

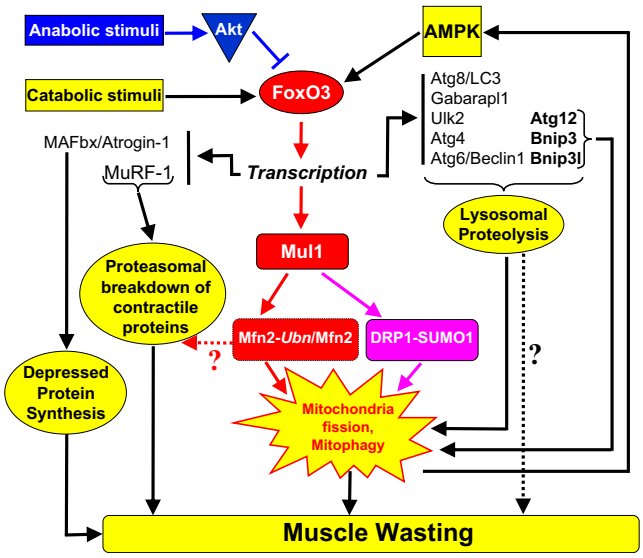

Figure 1. FoxO3-Dependent Mitophagy

The findings of Lokireddy et al. (2012) are shown in red. Under anabolic stimuli (in blue), Akt phosphorylates FoxO3, leading to its exclusion from the nucleus and inhibition of transcription. Under catabolic stimuli (in yellow), dephosphorylated FoxO3 stimulates the transcription of MuRF-1 and MAFbx/atrogin-1, which are involved in the proteasome-dependent breakdown of contractile proteins and in depressed protein synthesis, respectively. Dephosphorylated FoxO3 also enhances the transcription of autophagy-related genes (Atg8/LC3, Gabarapl1, Ulk2, Atg4, Atg6/Beclin1, Atg12, Bnip3, and Bnip3l). Genes involved in mitochondria fission are shown in bold (Romanello et al., 2010). Romanello et al. also reported that AMPK activation is crucial for mitophagy and concluded that the mitochondrial network is an essential amplificatory loop of the muscular atrophy program. Note that Mul1 is involved in the downregulation of mitochondrial fusion (through Mfn2 ubiquitination) and increased mitochondrial fission (through DRP1 sumoylation, see Braschi et al. [2009]). Mfn2-*Ubn*/Mfn2 represents the ratio of polyubiquitinated Mfn2/Mfn2. Dotted lines and ? denote hypotheses that need to be experimentally addressed.

A number of questions remain (see Figure 1). It is not clear whether the lysosomal pathway also degrades key muscle proteins in addition to its regulatory role in mitochondrial fission and subsequent muscle wasting. Mul1-induced mitophagy was previously reported to involve the Mul1-dependent sumoylation of the mitochondrial fission GTPase dynamin-related protein 1 (DRP1) (see Braschi et al. [2009]). The respective roles of polyubiquitination (this paper) and sumoylation of the targets of Mul1 in mitochondrial fusion and fission thus remain to be explored. Whether Mul1 has any direct effect on the UPS and contributes to the catabolic response must be further investigated. Finally, muscle wasting results not only from a coordinate activation of the UPS and the lysosomal pathway, but also from the activation of the Ca^{2+}-dependent and caspase systems and matrix metalloproteases (for the latter activation in a model of immobilization-induced atrophy, see Slimani et al. [2012]). Therefore, a first important goal would be to precisely identify proteins degraded by each proteolytic system under muscle-wasting conditions. Second, and more importantly, determining whether all muscle proteolytic systems are coordinately activated, and whether activation requires several transcription factors or one such as FoxO3, is a forthcoming challenge. Altogether, these experiments should help the design of new and effective approaches to prevent or limit muscle wasting.

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